



In re Application of: Akira ASAKURA, et al.
Serial No.: 09/470,667
For: NOVEL ALCOHOL/ALDEHYDE
DEHYDROGENASES

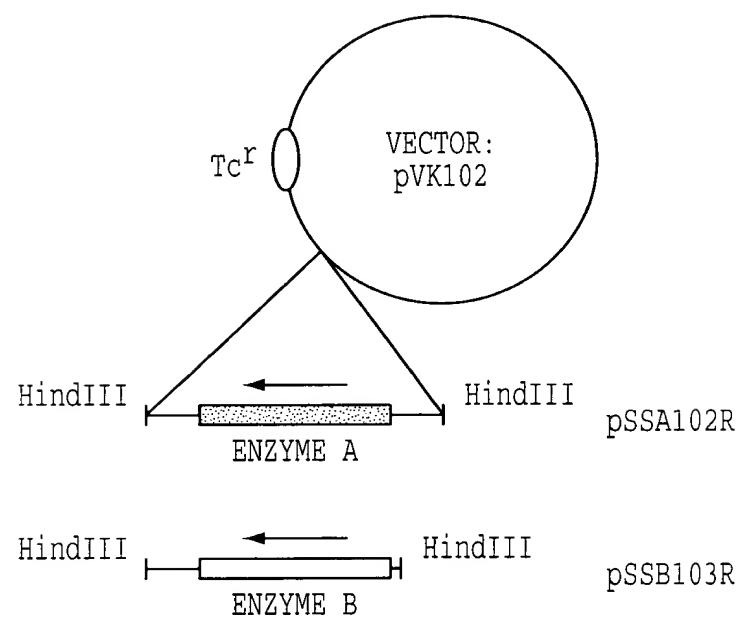
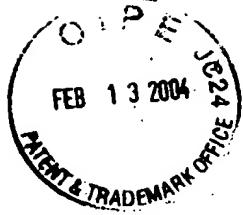


FIG. 1



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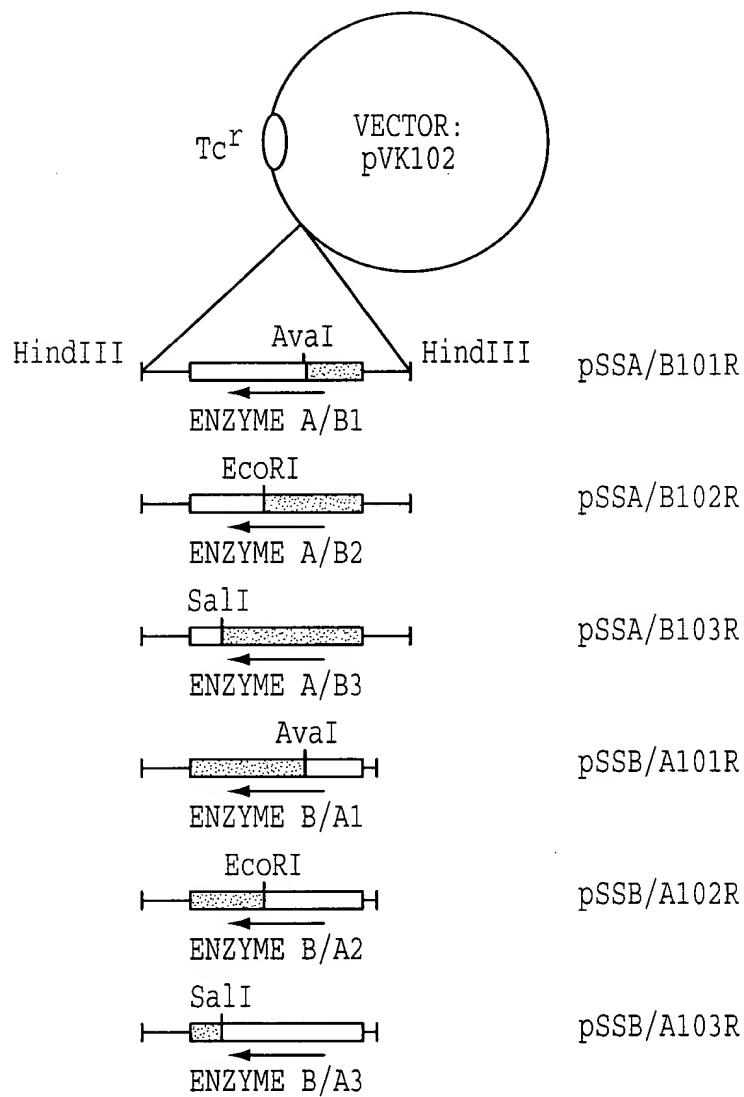
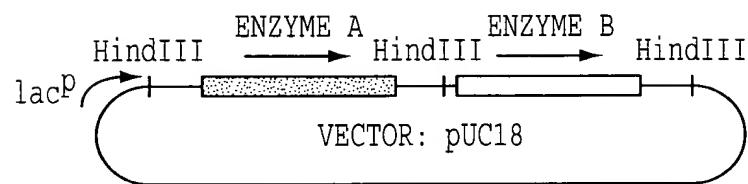


FIG. 2



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pSSAB201



pSSBA201

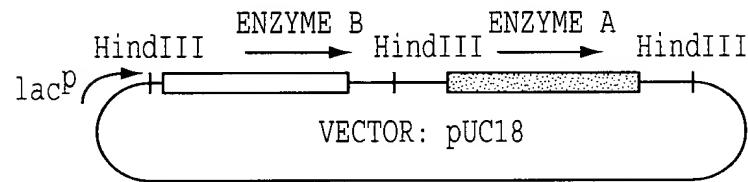
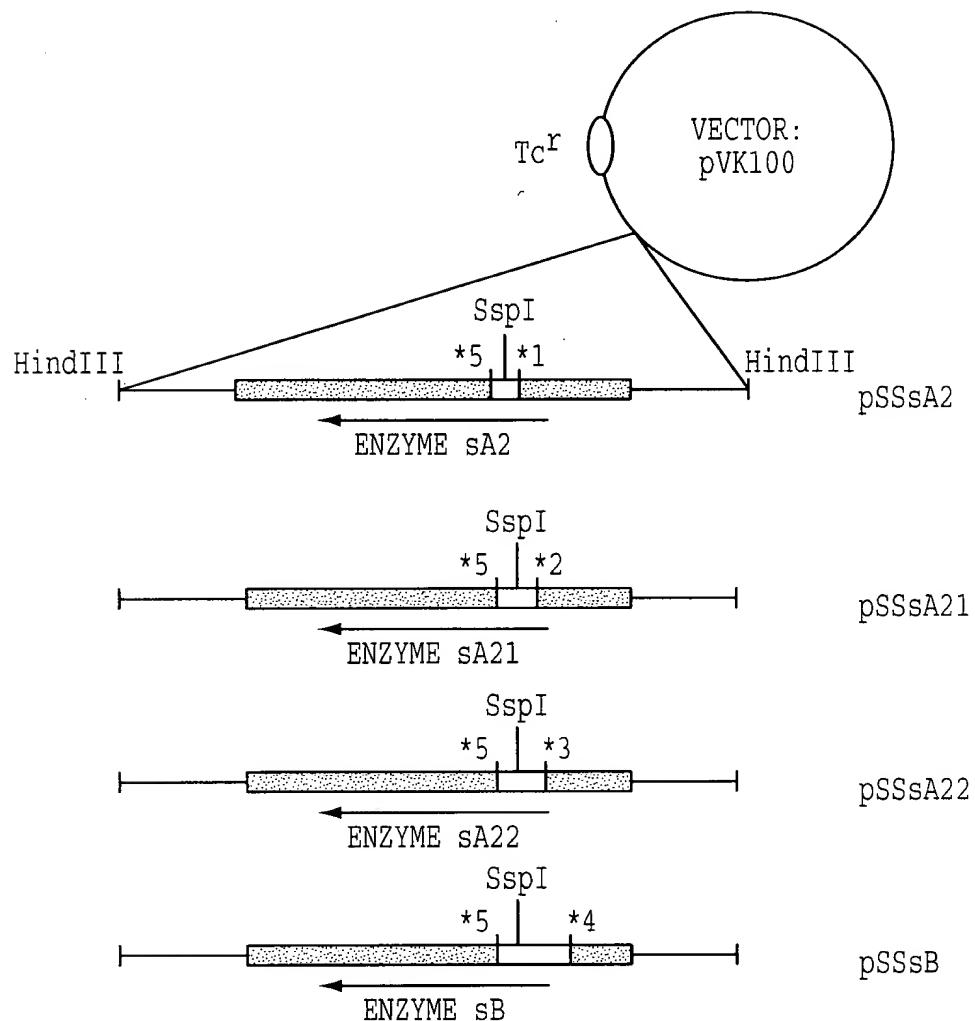


FIG. 3



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RECOMBINATION SITE

- *1 : AMINO ACID RESIDUE NO. 135 OF MATURE ENZYME A
- *2 : AMINO ACID RESIDUE NO. 128 OF MATURE ENZYME A
- *3 : AMINO ACID RESIDUE NO. 125 OF MATURE ENZYME A
- *4 : AMINO ACID RESIDUE NO. 95 OF MATURE ENZYME A
- *5 : AMINO ACID RESIDUE NO. 180 OF MATURE ENZYME B,
 WHICH NUCLEOTIDE SEQUENCE OF AvaI SITE ENCODES

FIG. 4



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ENZYME A 1 : QVTPVTDELL ANPPAGEWIS YGQNQENYRH SPLTQITTEN VGQLQLVWAR GMQPGKVQVT
ENZYME B 1 : QVTPITDELL ANPPAGEWIN YGRNQENYRH SPLTQITADM VGQLQLVWAR GMEAGAVQVT

61 : PLIHDGVMYL ANPGDVIQAI DAKTGDLIWE HRRQLPNIAT LNSFGEPTRG MALYGTNVYF
*
61 : PMIHDGVMYL ANPGDVIOAL DAOTGDLIWE HRROLPAVAT LNAOGDRKRG VALYGTSLYF

* Avail

121 : SSWDNHLIAL DMETGQVVFD VERGSGEDGL TSNTTGPIVA NGVIVAGSTC QYSPYGCFIS

181 : GHDSATGEEL WRNHFIPQPG EEGDETWGND FEARWMTGVW GQITYDPVTN LVFYGSTGVG

241 : PASETQRGTP GGTLYGTNTR FAVRPDTGEI VWRHQTLPRD NWDQECTFEM MVANVDVQPS
*

EcoRI

301 : AEMEGLRAIN PNAATGERRV LTGAPCKTGT MWSFDAASGE FLWARDTNYT NMIASIDETG

361 : LVTVNEDAVL KELDVYDVC PTELGGRDWS SAALNPDTGI YFLPLNNACY DIMAVDOEFS

Sali

421 : ALDVYNTSAT AKLAPGFENM GRIDAIIDIST GRTLWSAERP AANYSPVLST AGGVVFNGGT

481 : DRYFRALSOE TGETLWOARL ATVATGOAIS YELDGVOYIA IGAGGLTYGT OLNAPLA-EA

540 : VDSTAIGNAV YVFALPQ
 *** *** *****

540 : IDSTSVGNAI YVFALPQ

* : NUCLEOTIDE SEQUENCES ENCODING THESE REGIONS ARE THE RESTRICTION SITES FOR AvaI, EcoRI, AND SalI WHICH WERE USED FOR CONSTRUCTING CHIMERA GENES SHOWN IN FIG. 2.

FIG. 5

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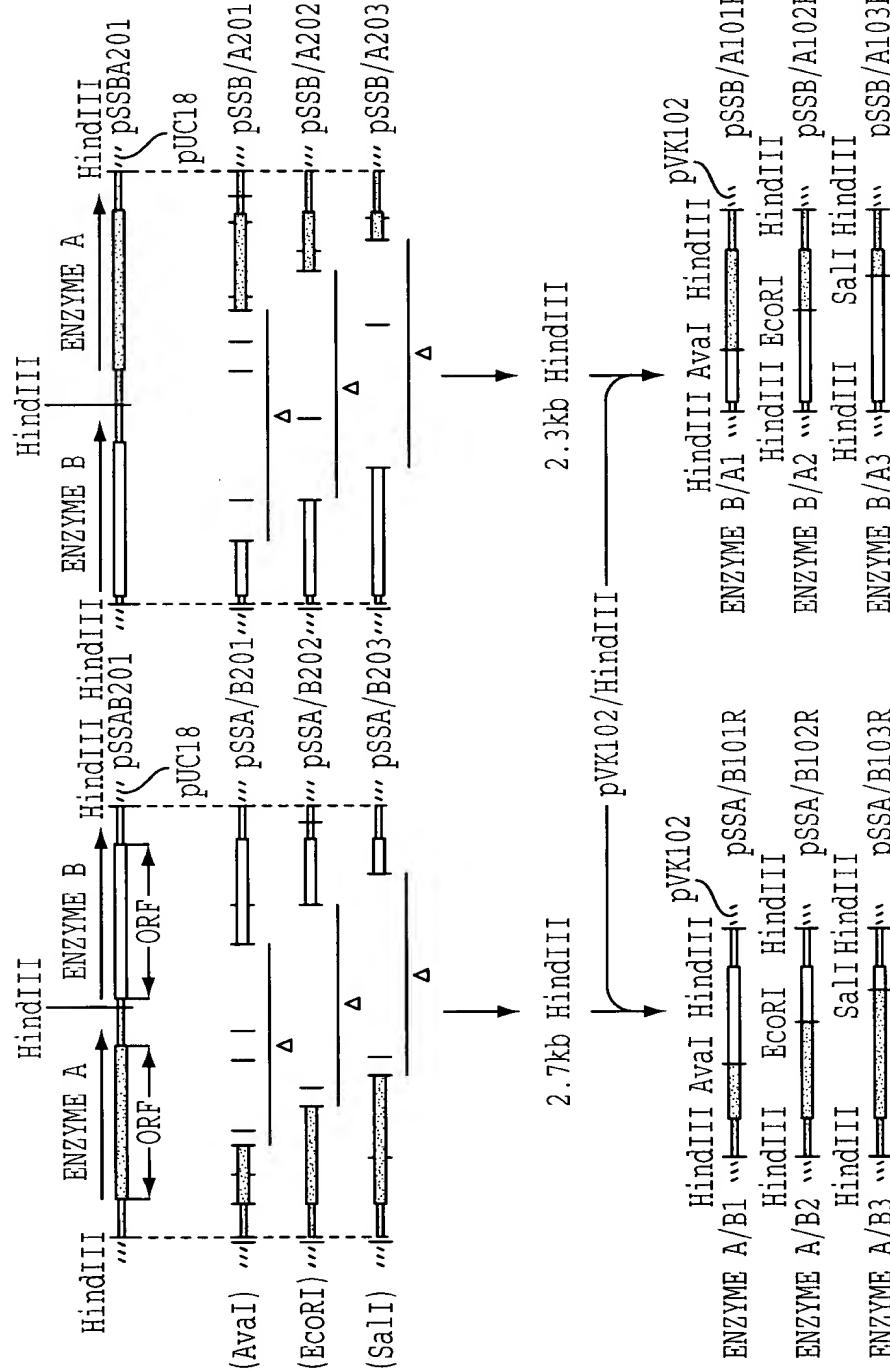
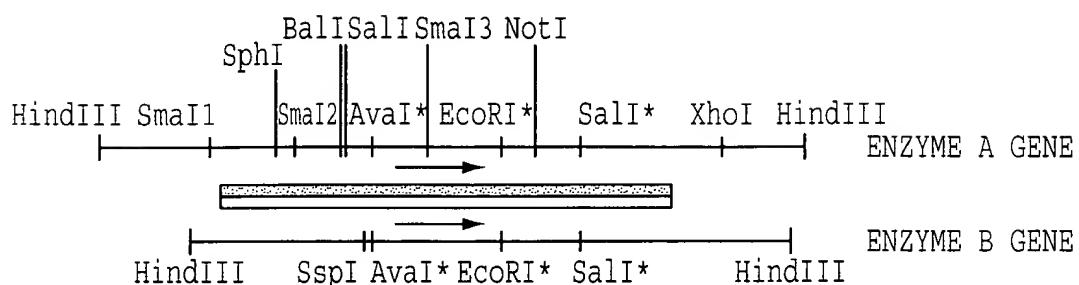


FIG. 6

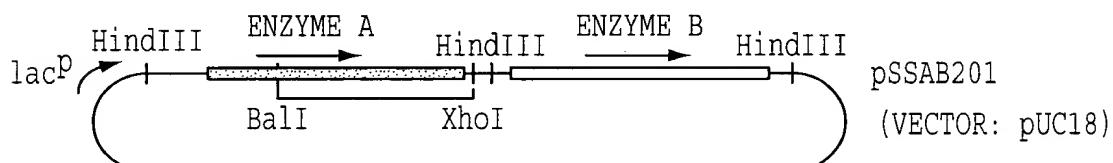


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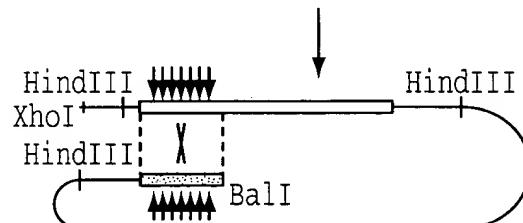


*: AvaI, EcoRI, SalI SITES USED FOR CONSTRUCTING CHIMERA GENES SHOWN IN FIG. 2 AND 6.

FIG. 7



LINEARLIZATION WITH XhoI AND BalI



TRANSFORM *E. coli* JM101 (rec A+)

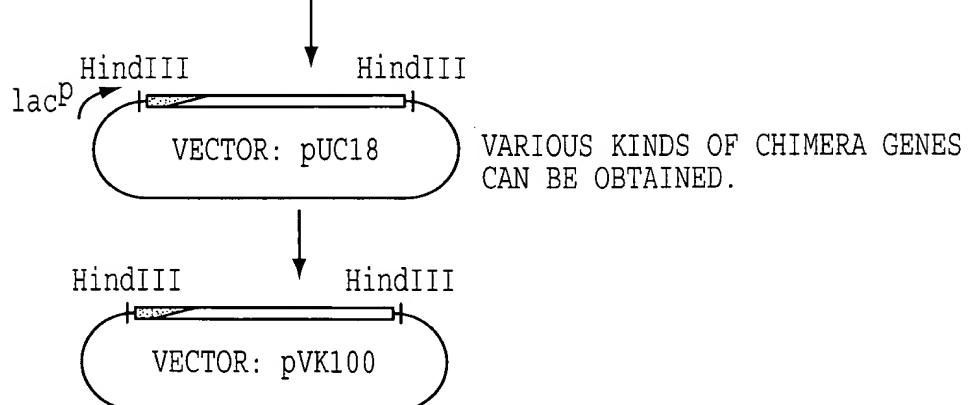


FIG. 8



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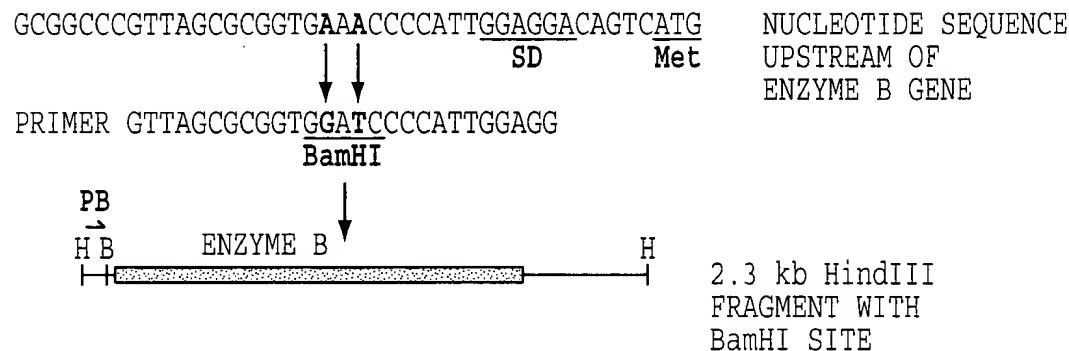


FIG. 9

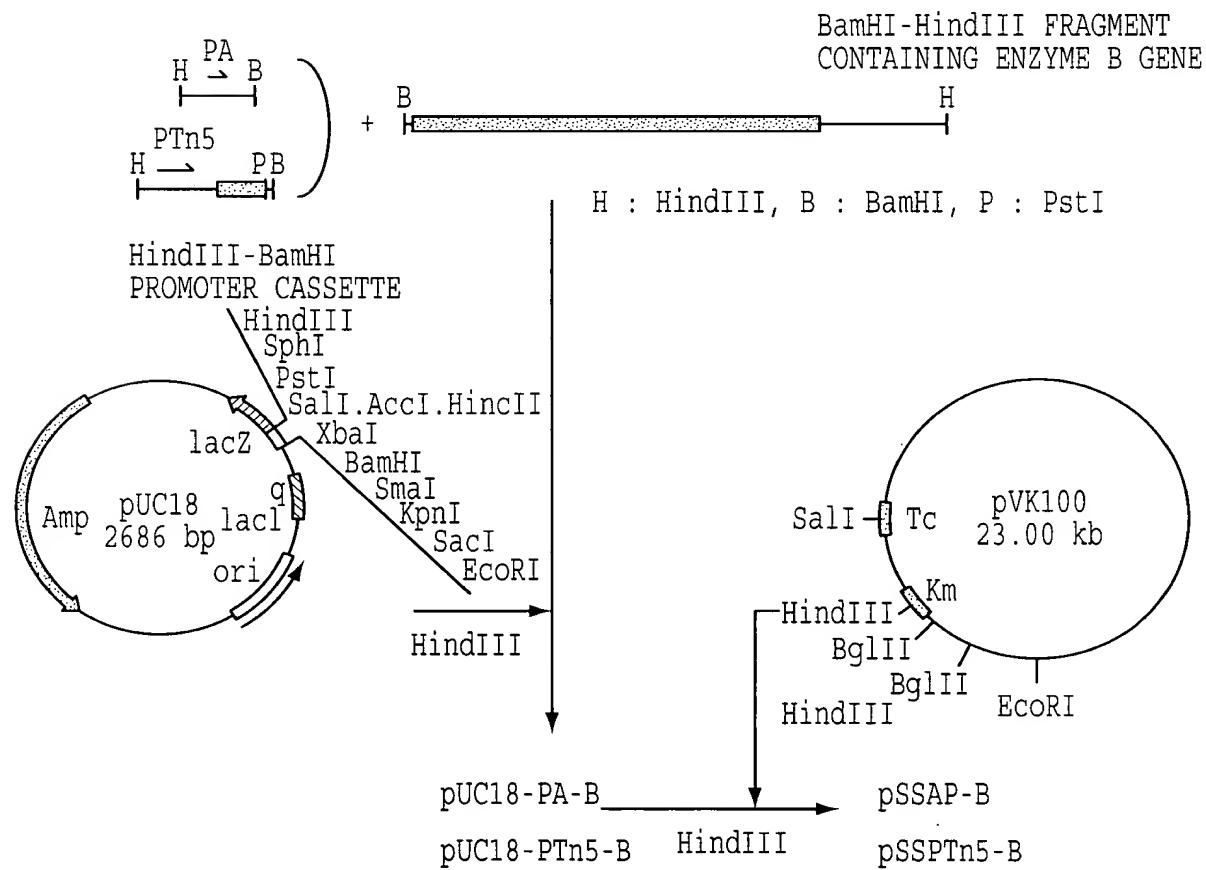
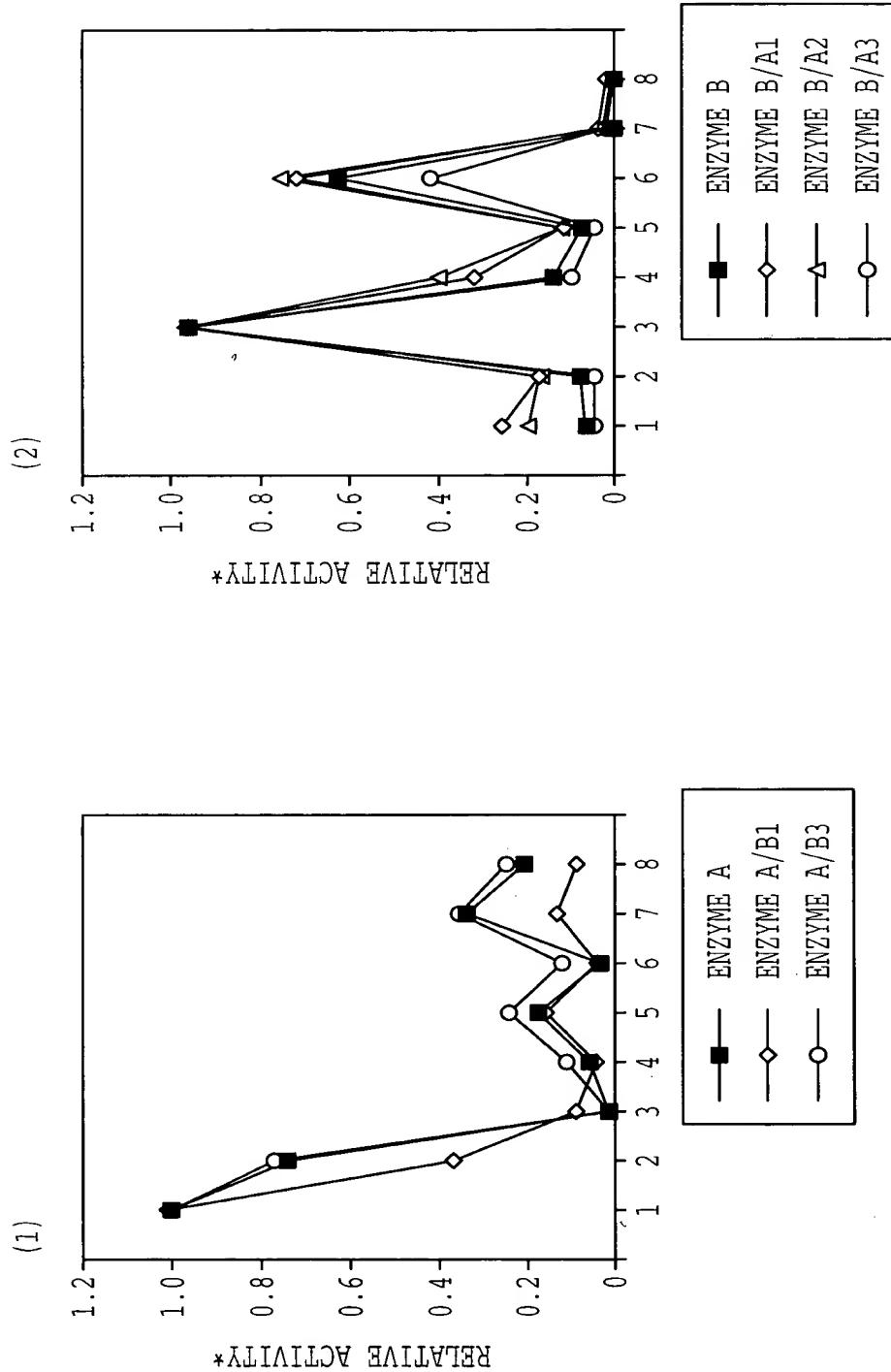


FIG. 10

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1. n-PROPANOL
2. ISOPROPANOL
3. D-GLUCOSE
4. L-SORBOSONE
5. D-SORBITOL
6. D-MANNITOL
7. L-SORBOSE
8. D-FRUCTOSE

*ENZYME ACTIVITY WAS NORMALIZED RELATIVE TO ACTIVITY FOR n-PROPANOL (1), OR D-GLUCOSE (2).
ENZYME A/B2 WAS EXCEPTED BECAUSE OF ITS LOW EXPRESSION IN *P. putida*.

FIG. 11